IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Olsen *et al.*	Art Unit: *To be assigned*
Appl. No. *To be assigned* (Divisional of U.S. Appl. No. 08/994,962; Filed: December 19, 1997)	Examiner: *To be assigned*
Filed: *Herewith*	Atty. Docket: 1488.0440003/EKS/PSC
For: **Human Oncogene Induced Secreted Protein I**	

First Preliminary Amendment

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In advance of prosecution, please amend the application as follows. This Amendment is provided in the following format:

(A) A clean version of each replacement paragraph/section/claim along with clear instructions for entry;

(B) Starting on a separate page, appropriate remarks and arguments. 37 C.F.R. § 1.115; and

(C) Starting on a separate page, a marked-up version entitled: "Version with markings to show changes made."

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

Amendments

Please amend the application as follows.

In the Specification:

Please substitute the paragraph beginning at page 1, line 4 with the following paragraph:

The present application is a divisional of United States Appl. No. 08/994,962, filed December 19, 1997 (now allowed), which is hereby incorporated by reference, said 08/994,962 claims priority benefit to provisional United States Appl. No. 60/033,869, filed December 20, 1996, which is hereby incorporated by reference and provisional United States Appl. No. 60/037,388, filed February 7, 1997, which is hereby incorporated by reference.

Please substitute the paragraph beginning at page 12, line 24, with the following paragraph:

In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a portion of the polynucleotide in a nucleic acid molecule of the invention as described above, for instance, the cDNA clone deposited with the ATCC on December 16, 1996 (ATCC Deposit No. 97825). By "stringent hybridization conditions" is intended overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 μg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

In the Claims

Please cancel claims 1-16

Please add the following new claims.

17. An isolated protein comprising an amino acid sequence at least 90% identical to amino acids 1 to 142 of SEQ ID NO:2.

18. The protein of claim 17, comprising an amino acid sequence at least 95% identical to amino acids 1 to 142 of SEQ ID NO:2.

19. The protein of claim 18, comprising amino acids 1 to 142 of SEQ ID NO:2.

20. The protein of claim 17, comprising an amino acid sequence at least 90% identical to amino acids -19 to 142 of SEQ ID NO:2.

21. The protein of claim 20, comprising an amino acid sequence at least 95% identical to amino acids -19 to 142 of SEQ ID NO:2.

22. The protein of claim 21, comprising amino acids -19 to 142 of SEQ ID NO:2.

23. The protein of claim 20, comprising an amino acid sequence at least 90% identical to amino acids -20 to 142 of SEQ ID NO:2.

24. The protein of claim 23, comprising an amino acid sequence at least 95% identical to amino acids -20 to 142 of SEQ ID NO:2.

25. The protein of claim 24, comprising amino acids -20 to 142 of SEQ ID NO:2.

26. The protein of claim 17, which is produced by a recombinant host cell.

27. The protein of claim 17, which comprises a heterologous protein.

28. A composition comprising the protein of claim 17 and a pharmaceutically acceptable carrier.

29. A method of producing the protein of claim 17, comprising:

(a) culturing a host cell under conditions suitable to produce the protein; and

(b) recovering the protein from the cell culture.

30. A protein produced by a method comprising:

(a) expressing the protein of claim 17 in a cell; and

(b) recovering said protein.

31. An isolated protein comprising, except for one to five conservative amino acid substitutions, an amino acid sequence identical to amino acids 1 to 142 of SEQ ID NO:2.

32. The protein of claim 31 comprising, except for one to five conservative amino acid substitutions, an amino acid sequence identical to amino acids -19 to 142 of SEQ ID NO:2.

33. The protein of claim 32 comprising, except for one to five conservative amino acid substitutions, an amino acid sequence identical to amino acids -20 to 142 of SEQ ID NO:2.

34. The protein of claim 31, which is produced by a recombinant host cell.

35. The protein of claim 31, which comprises a heterologous protein.

36. A composition comprising the protein of claim 31 and a pharmaceutically acceptable carrier.

37. A method of producing the protein of claim 31, comprising:

(a) culturing a host cell under conditions suitable to produce the protein; and

(b) recovering the protein from the cell culture.

38. A protein produced by a method comprising:

(a) expressing the protein of claim 31 in a cell; and

(b) recovering said protein.

39. An isolated protein, comprising an amino acid sequence at least 90% identical to the mature amino acid sequence encoded by the cDNA in ATCC Deposit No. 97825.

40. The protein of claim 39, comprising an amino acid sequence at least 95% identical to the mature amino acid sequence encoded by the cDNA in ATCC Deposit No. 97825.

41. The protein of claim 40, comprising the mature amino acid sequence encoded by the cDNA in ATCC Deposit No. 97825.

42. The protein of claim 39, comprising an amino acid sequence at least 90% identical to the complete amino acid sequence encoded by the cDNA in ATCC Deposit No. 97825.

43. The protein of claim 42, comprising an amino acid sequence at least 95% identical to the complete amino acid sequence encoded by the cDNA in ATCC Deposit No. 97825.

44. The protein of claim 43, comprising the complete amino acid sequence encoded by the cDNA in ATCC Deposit No. 97825.

45. The protein of claim 39, which is produced by a recombinant host cell.

46. The protein of claim 39, which comprises a heterologous protein.

47. A composition comprising the protein of claim 39 and a pharmaceutically acceptable carrier.

48. A method of producing the protein of claim 39, comprising:

(a) culturing a host cell under conditions suitable to produce the protein; and

(b) recovering the protein from the cell culture.

49. A protein produced by a method comprising:

(a) expressing the protein of claim 39 in a cell; and

(b) recovering said protein.

50. An isolated protein comprising, except for one to five conservative amino acid substitutions, an amino acid sequence identical to the mature amino acid sequence encoded by the cDNA in ATCC Deposit NO. 97825.

51. The protein of claim 50, which is produced by a recombinant host cell.

52. The protein of claim 50, which comprises a heterologous protein.

53. A composition comprising the protein of claim 50 and a pharmaceutically acceptable carrier.

54. A method of producing the protein of claim 50, comprising:

(a) culturing a host cell under conditions suitable to produce the protein; and

(b) recovering the protein from the cell culture.

55. A protein produced by a method comprising:

(a) expressing the protein of claim 50 in a cell; and

(b) recovering said protein.

56. An isolated protein comprising, except for one to five conservative amino acid substitutions, an amino acid sequence identical to the complete amino acid sequence encoded by the cDNA in ATCC Deposit No. 97825.

57. The protein of claim 56, which is produced by a recombinant host cell.

58. The protein of claim 56, which comprises a heterologous protein.

59. A composition comprising the protein of claim 56 and a pharmaceutically acceptable carrier.

60. A method of producing the protein of claim 56, comprising:

(a) culturing a host cell under conditions suitable to produce the protein; and

(b) recovering the protein from the cell culture.

61. A protein produced by a method comprising:

(a) expressing the protein of claim 56 in a cell; and

(b) recovering said protein.

62. An isolated protein comprising a fragment of at least 30 contiguous amino acids of SEQ ID NO:2, wherein said fragment comprises an amino acid sequence selected from the group consisting of:

(a) amino acids -4 to 9 of SEQ ID NO:2;

(b) amino acids 13 to 19 of SEQ ID NO:2;

(c) amino acids 23 to 32 of SEQ ID NO:2;

(d) amino acids 36 to 47 of SEQ ID NO:2;

(e) amino acids 54 to 63 of SEQ ID NO:2;

(f) amino acids 70 to 74 of SEQ ID NO:2;

(g) amino acids 90 to 100 of SEQ ID NO:2;

(h) amino acids 105 to 119 of SEQ ID NO:2; and

(i) amino acids 125 to 132 of SEQ ID NO:2.

63. The protein of claim 62, wherein said fragment comprises amino acid sequence (a).

64. The protein of claim 62, wherein said fragment comprises amino acid sequence (b).

65. The protein of claim 62, wherein said fragment comprises amino acid sequence (c).

66. The protein of claim 62, wherein said fragment comprises amino acid sequence (d).

67. The protein of claim 62, wherein said fragment comprises amino acid sequence (e).

68. The protein of claim 62, wherein said fragment comprises amino acid sequence (f).

69. The protein of claim 62, wherein said fragment comprises amino acid sequence (g).

70. The protein of claim 62, wherein said fragment comprises amino acid sequence (h).

71. The protein of claim 62, wherein said fragment comprises amino acid sequence (i).

72. The protein of claim 62, which is produced by a recombinant host cell.

73. The protein of claim 62, which comprises a heterologous protein.

74. A composition comprising the protein of claim 62 and a pharmaceutically acceptable carrier.

75. A method of producing the protein of claim 62, comprising:

(a) culturing a host cell under conditions suitable to produce the protein; and

(b) recovering the protein from the cell culture.

76. A protein produced by a method comprising:

(a) expressing the protein of claim 62 in a cell; and

(b) recovering said protein.

77. An isolated protein comprising 30 contiguous amino acids of SEQ ID NO:2.

78. The protein of claim 77, comprising 50 contiguous amino acids of SEQ ID NO:2.

79. The protein of claim 77, which is produced by a recombinant host cell.

80. The protein of claim 77, which comprises a heterologous protein.

81. A composition comprising the protein of claim 77 and a pharmaceutically acceptable carrier.

82. A method of producing the protein of claim 77, comprising:

(a) culturing a host cell under conditions suitable to produce the protein; and

(b) recovering the protein from the cell culture.

83. A protein produced by a method comprising:

(a) expressing the protein of claim 77 in a cell; and

(b) recovering said protein.

84. An isolated protein comprising an amino acid sequence at least 90% identical to a fragment of SEQ ID NO:2, wherein said fragment is at least 30 amino acids in length.

85. The protein of claim 84, comprising an amino acid sequence at least 95% identical to said fragment.

86. The protein of claim 85, comprising an amino acid sequence identical to said fragment.

87. The protein of claim 84, wherein said fragment is at least 50 amino acids in length.

88. The protein of claim 84, which is produced by a recombinant host cell.

89. The protein of claim 84, which comprises a heterologous protein.

90. A composition comprising the protein of claim 84 and a pharmaceutically acceptable carrier.

91. A method of producing the protein of claim 84, comprising:

(a) culturing a host cell under conditions suitable to produce the protein; and

(b) recovering the protein from the cell culture.

92. A protein produced by a method comprising:

(a) expressing the protein of claim 84 in a cell; and

(b) recovering said protein.

93. An isolated protein comprising an amino acid sequence at least 90% identical to a fragment of the amino acid sequence encoded by the cDNA in ATCC Deposit No. 97825, wherein said fragment is at least 30 amino acids in length.

94. The protein of claim 93, comprising an amino acid sequence at least 95% identical to said fragment.

95. The protein of claim 94, comprising an amino acid sequence identical to said fragment.

96. The protein of claim 93, wherein said fragment is at least 50 amino acids in length.

97. The protein of claim 93, which is produced by a recombinant host cell.

98. The protein of claim 93, which comprises a heterologous protein.

99. A composition comprising the protein of claim 93 and a pharmaceutically acceptable carrier.

100. A method of producing the protein of claim 93, comprising:

(a) culturing a host cell under conditions suitable to produce the protein; and

(b) recovering the protein from the cell culture.

101. A protein produced by a method comprising:

(a) expressing the protein of claim 93 in a cell; and

(b) recovering said protein.

102. An isolated protein comprising an amino acid sequence encoded by a polynucleotide which hybridizes to a nucleic acid encoding SEQ ID NO:2, or the complement thereof; or a nucleic acid encoded by the cDNA in ATCC Deposit No. 97825, or the complement thereof, under conditions comprising:

(a) incubating overnight at 42° C in a solution consisting of 50% formamide, 5X SSC, 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20μg/ml denatured, sheared salmon sperm DNA; and

(b) washing at 65° C in a solution consisting of 0.1x SSC.

103. The protein of claim 102, wherein said polynucleotide hybridizes to a nucleic acid encoding SEQ ID NO:2, or the complement thereof, under said conditions.

104. The protein of claim 102, wherein said polynucleotide hybridizes to a nucleic acid encoded by the cDNA in ATCC Deposit No. 97825, or the complement thereof, under said conditions.

105. The protein of claim 102, which is produced by a recombinant host cell.

106. The protein of claim 102, which comprises a heterologous protein.

107. A composition comprising the protein of claim 102 and a pharmaceutically acceptable carrier.

108. A method of producing the protein of claim 102, comprising:

(a) culturing a host cell under conditions suitable to produce the protein; and

(b) recovering the protein from the cell culture.

109. A protein produced by a method comprising:

(a) expressing the protein of claim 102 in a cell; and

(b) recovering said protein.

110. An isolated protein comprising 30 contiguous amino acids of the complete amino acid sequence encoded by the cDNA in ATCC Deposit No. 97825.

111. The protein of claim 110, comprising 50 contiguous amino acids of the complete amino acid sequence encoded by the cDNA in ATCC Deposit No. 97825.

112. The protein of claim 110, which is produced by a recombinant host cell.

113. The protein of claim 110, which comprises a heterologous protein.

114. A composition comprising the protein of claim 110 and a pharmaceutically acceptable carrier.

115. A method of producing the protein of claim 110, comprising:

(a) culturing a host cell under conditions suitable to produce the protein; and

(b) recovering the protein from the cell culture.

116. A protein produced by a method comprising:

(a) expressing the protein of claim 110 in a cell; and

(b) recovering said protein.

Remarks

The specification has been amended in order to incorporate the proper priority information for the captioned application. In addition, the specification has been amended to correct inadvertent typographical errors. Specifically, the specification has also been amended to correct obvious typographical errors in the amount of ingredients listed for 5x SSC (sodium chloride/sodium citrate) and the concentration of salmon sperm DNA used in stringent hybridization. An amendment to correct an obvious error does not constitute new matter where one skilled in the art would not only recognize the existence of the error in the specification, but also the appropriate correction. (M.P.E.P. 2163.07) Here, the recognition of the typographical errors, along with the correction of the errors, in the amount of the ingredients listed for 5x SSC and in the concentration of salmon sperm DNA used in the hybridization, are obvious to one skilled in the art, and therefore, the correction does not constitute new matter. A 5x SSC is a well known solution used in hybridization solutions. *See*, e.g., Exhibit A, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley and Sons, N.Y., page 2.10.7 (1987). SSC is normally made as a 20x stock solution, and then diluted accordingly for a particular use. Exhibit B shows that a 20x SSC stock solution contains 3M NaCl and 0.3M trisodium citrate. (Exhibit B, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley and Sons, N.Y., page A.2.5. (1987)). To make a 5x SSC solution, the 20x SSC solution must be diluted by one-forth. Therefore, a 5x SSC solution contains 750mM NaCl (3M ÷ 4 = 750mM) and 75mM trisodium citrate (0.3M ÷ 4 = 75mM).

One skilled in the art would have immediately recognized that the amount of ingredients listed in the specification for a 5x SSC solution was incorrect. Rather than describing a 5x SSC solution, made up of 750mM NaCl and 75mM trisodium citrate, the specification inaccurately listed the ingredients for a 1x SSC solution. The skilled artisan, in recognizing the typographical error, could easily have adjusted the amount of ingredients described in the specification to properly make a 5x SSC solution.

Likewise, the relative amount of salmon sperm DNA to be used in a hybridization is well known (See, *e.g.*, Exhibit A). Exhibit A shows that a hybridization solution typically contains about 100 μg/ml salmon sperm DNA. One skilled in the art would have immediately recognized that the

concentration of salmon sperm DNA was incorrect in the specification, as it recited 20 g/ml, an exponentially larger concentration than 100 µg/ml. The skilled artisan, in recognizing the typographical error, could have adjusted the concentration of salmon sperm DNA accordingly.

Therefore, because no new matter will be added to the specification if these typographical errors are corrected, Applicants respectfully request that the amendments to the specification to recite the correct amount of ingredients for a 5x SSC solution and concentration of salmon sperm DNA be entered.

Claims 1-16 have been cancelled without prejudice or disclaimer of the subject matter therein. Applicants reserve the right to pursue the subject matter of the cancelled claims in continuing applications. Claims 17-116 have been newly added. Support for the claims can be found throughout the specification and original claims. Specifically, support for claims 17-25 and 39-44 can be found, *inter alia*, at page 23, line 4 to page 24, line 9. Support for claims 31-33, 50 and 56 can be found, *inter alia*, at page 21, line 1 to page 22, line 11. Support for claims 62-71 can be found, *inter alia*, at page 25, lines 11-24. Support for claims 77-78 and 110-111 can be found, *inter alia*, at page 20, lines 13-16 and page 23, lines 12-13. Support for claims 84-87 and 93-96 can be found, *inter alia*, at page 20, lines 13-16; page 21, lines 1-14; and page 23, lines 12-13. Support for claims 102-104 can be found, *inter alia*, at page 12, line 24 to page 13, line 2. Support for claims 26-27, 29-30, 34-35, 37-38, 45-46, 48-49, 51-52, 54-55, 57-58, 60-61, 72-73, 75-76, 79-80, 82-83, 88-89, 91-92, 97-98, 100-101, 105-106, 108-109, 112-113, and 115-116 can be found, *inter alia*, at page 17, line 20 to page 20, line 11. Support for claims 28, 36,47, 53, 59, 74, 81, 90, 99, 107, and 114 can be found, *inter alia*, at page 31, lines 1-26.

Upon entry of the forgoing amendments, claims 17-116 are pending in the application with claims 17, 31, 39, 50, 56, 62, 77, 84, 93, 102, and 110 being the independent claims.

Conclusion

Applicants believe that this application is now in condition for substantive examination.

Early notice to this effect is respectfully requested. The U.S. Patent and Trademark Office is hereby authorized to charge any fee deficiency, or credit any overpayment, to our Deposit Account No. 19-0036.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Reg. No. 47,473

for Eric K. Steffe
Attorney for Applicants
Registration No. 36,688

Date: July 9, 2001

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Versions with Markings to show changes made

In the claims:

Claims 1-16 have been cancelled.

Claims 17-116 have been newly added.

In the Specification:

The paragraph beginning at page 1, line 4:

The present application is a divisional of United States Appl. No. 08/994,962, filed December 19, 1997 (now allowed), which is hereby incorporated by reference, said 08/994,962 [This application hereby] claims priority benefit to provisional United States Appl. No. 60/033,869, filed December 20, 1996, which is hereby incorporated by reference and provisional United States Appl. No. 60/037,388, filed February 7, 1997, which [are]is hereby incorporated by reference.

The paragraph beginning at page 12, line 24:

In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a portion of the polynucleotide in a nucleic acid molecule of the invention as described above, for instance, the cDNA clone deposited with the ATCC on December 16, 1996 (ATCC Deposit No. 97825). By "stringent hybridization conditions" is intended overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC ([150]750 mM NaCl, [15]75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Olsen *et al.* Appl. No. *To be assigned* (Divisional of U.S. Appl. No. 08/994,962; Filed December 19, 1997) Filed: *Herewith* For: **Human Oncogene Induced Secreted Protein I**	Confirmation No.: N/A Art Unit: *To be assigned* Examiner: *To be assigned* Atty. Docket: 1488.0440003/EKS/PSC

Second Preliminary Amendment

Commissioner for Patents
Washington, D.C. 20231

Sir:

In advance of prosecution, please amend the application as follows. This Amendment is provided in the following format:

(A) A clean version of each replacement paragraph/section/claim along with clear instructions for entry;

(B) Starting on a separate page, appropriate remarks and arguments. 37 C.F.R. § 1.115; and

(C) Starting on a separate page, a marked-up version entitled: "Version with markings to show changes made."

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

Amendments

In the Specification:

Please substitute the following paragraphs/sections for the pending paragraphs/sections.

The paragraph beginning at page 5, line 2:

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding the HOIPS I polypeptide having the amino acid sequence shown in FIGs 1A-1B (SEQ ID NO:2) or the amino acid sequence encoded by the cDNA clone deposited in a bacterial host with the American Type Culture Collection ("ATCC"), Patent Depository, 10801 University Boulevard, Manassas, VA, 20110-2209, on December 16, 1996. (ATCC Deposit Number 97825).

The paragraph beginning at page 6, line 8:

FIGs 1A-1B show the nucleotide (SEQ ID NO:1) and deduced amino acid (SEQ ID NO:2) sequences of HOIPS I. The protein has a leader sequence of about 20 amino acid residues and a deduced molecular weight of about 17.8 kDa. The predicted amino acid sequence of the mature HOIPS I protein is also shown in FIGs 1A-1B (SEQ ID NO:2).

The paragraph beginning at page 6, line 13:

FIG. 2 shows the regions of similarity between the amino acid sequences of the HOIPS I protein and chicken MD-1 (SEQ ID NO:3). The consensus sequence is shown (SEQ ID NO:17).

The paragraph beginning at page 6, line 16:

FIG. 3 shows an analysis of the HOIPS I amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown. In the "Antigenic Index - Jameson-Wolf" graph, amino acid residues about 17 to about 29, about 33 to about 39, about 43 to about 52, about 56 to about 67, about 74 to about 83, about 90 to about 94, about 110 to about 120, about 125 to about 139, and about 145 to about 152 in FIGs 1A-1B correspond to the shown highly antigenic regions of the HOIPS I protein. These highly antigenic fragments in FIGs 1A-1B correspond to the following fragments, respectively in SEQ ID NO:2: amino acid residues about -4 to about 9, about 13 to about 19, about 23 to about 32, about 36 to about 47, about 54 to about 63, about 70 to about 74, about 90 to about 100, about 105 to about 119, and about 125 to about 132.

The paragraph beginning at page 7, line 2:

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding a HOIPS I polypeptide having the amino acid sequence shown in FIGs 1A-1B (SEQ ID NO:2), which was determined by sequencing a cloned cDNA. The HOIPS I protein of the present invention shares sequence homology with the chicken MD-1 protein. (FIG. 2) (SEQ ID NO:3). The nucleotide sequence shown in FIGs 1A-1B (SEQ ID NO:1) was obtained by sequencing the HTOCD71X clone, which was deposited on December 16, 1996 at the American Type Culture Collection, Patent Depository, 10801 University Boulevard, Manassas, VA, 20110-2209. (ATCC accession number 97825) The deposited clone is contained in the pBluescript SK(-) plasmid (Stratagene, LaJolla, CA).

The paragraph beginning at page 8, line 3:

Using the information provided herein, such as the nucleotide sequence in FIGs 1A-1B, a nucleic acid molecule of the present invention encoding a HOIPS I polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material. Illustrative of the invention, the nucleic acid molecule described in FIGs 1A-1B (SEQ ID NO:1) was discovered in a cDNA library derived from human tonsils tissue. The gene was also identified in cDNA libraries from the following tissues: bone marrow, dendritic cells, fetal and adult brain macrophages, B cells, and lymph nodes. The determined nucleotide sequence of the HOIPS I cDNA of FIGs 1A-1B (SEQ ID NO:1) contains an open reading frame encoding a protein of 162 amino acid residues and a deduced molecular weight of about 17.8 kDa. The HOIPS I protein shown in FIGs 1A-1B (SEQ ID NO:2) is about 45% identical to, and about 64% similar to, the chicken MD-1 protein (FIG. 2) in a 132 amino acid residue overlap.

The paragraph beginning at page 8, line 17:

The present invention also provides the mature form(s) of the HOIPS I protein of the present invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species on the protein. Further, it has long been known that the cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the

amino acid sequence of the polypeptide. Therefore, the present invention provides a nucleotide sequence encoding the mature HOIPS I polypeptides having the amino acid sequence encoded by the cDNA clone contained in the host deposited with the ATCC on December 16, 1996, (ATCC Deposit No. 97825) and as shown in FIGs 1A-1B (SEQ ID NO:2). By the mature HOIPS I protein having the amino acid sequence encoded by the cDNA clone contained in the host deposited with the ATCC on December 16, 1996, (ATCC Deposit No. 97825) is meant the mature form(s) of the HOIPS I protein produced by expression in a mammalian cell (e.g., COS cells, as described below) of the complete open reading frame encoded by the human DNA sequence of the clone contained in the vector in the deposited host. As indicated below, the mature HOIPS I having the amino acid sequence encoded by the cDNA clone contained in the host deposited with the ATCC on December 16, 1996, (ATCC Deposit No. 97825) may or may not differ from the predicted "mature" HOIPS I protein shown in FIGs 1A-1B (amino acids from about 1 to about 142 in SEQ ID NO:2) depending on the accuracy of the predicted cleavage site based on computer analysis.

The paragraph beginning at page 9, line 19:

In the present case, the predicted amino acid sequence of the complete HOIPS I polypeptides of the present invention were analyzed by a computer program ("PSORT") (K. Nakai and M. Kanehisa, *Genomics 14*:897-911 (1992)), which is an expert system for predicting the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated. The analysis by the PSORT program predicted the cleavage sites between amino acids 20 and 21 in FIGs 1A-1B (SEQ ID NO:2). Thereafter, the complete amino acid sequences were further analyzed by visual inspection, applying

a simple form of the (-1,-3) rule of von Heinje. von Heinje, *supra*. Thus, the leader sequence for the HOIPS I protein is predicted to consist of amino acid residues -20 to -1 in SEQ ID NO:2. However, while the predicted mature HOIPS I protein consists of residues 1-142, the present inventors have identified other possible cleavage sites resulting in mature proteins having the following amino acid residues shown in SEQ ID NO:2: -7-142, -6-142, -5-142, -4-142, -3-142, -2-142, -1-142, 2-142, 3-142, 4-142, 5-142, 6-142, 7-142, 8-142, 9-142, 10-142, 11-142, 12-142, 13-142, 14-142.

The paragraph beginning at page 10, line 26:

Isolated nucleic acid molecules of the present invention include DNA molecules comprising an open reading frame (ORF) shown in FIGs 1A-1B (SEQ ID NO:1); DNA molecules comprising the coding sequence for the mature HOIPS I protein shown in FIGs 1A-1B (last 142 amino acids) (SEQ ID NO:2); and DNA molecules which comprise a sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode the HOIPS I protein. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate variants.

The paragraph beginning at page 11, line 3:

In another aspect, the invention provides isolated nucleic acid molecules encoding the HOIPS I polypeptide having an amino acid sequence as encoded by the cDNA clone contained in the plasmid deposited with the ATCC on December 16, 1996 (ATCC Deposit No. 97825). In a further embodiment, nucleic acid molecules are provided encoding the mature HOIPS I polypeptide or the

full-length polypeptide lacking the N-terminal methionine. The invention also provides an isolated nucleic acid molecule having the nucleotide sequence shown in FIGs 1A-1B (SEQ ID NO:1) or the nucleotide sequence of the HOIPS I cDNA contained in the above-described deposited clone, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated molecules, particularly DNA molecules, are useful as probes for gene mapping, by *in situ* hybridization with chromosomes, and for detecting expression of the HOIPS I gene in human tissue, for instance, by Northern blot analysis.

The paragraph beginning at page 11, line 16:

The present invention is further directed to fragments of the isolated nucleic acid molecules described herein. By a fragment of an isolated nucleic acid molecule having the nucleotide sequence of the deposited cDNA or the nucleotide sequence shown in FIGs 1A-1B (SEQ ID NO:1) is intended fragments at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt in length which are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments 50, 100, 150, 200, 250, 300, 350, 400, 450, or 500 nt in length are also useful according to the present invention as are fragments corresponding to most, if not all, of the nucleotide sequence of the deposited cDNA or as shown in FIGs 1A-1B (SEQ ID NO:1). By a fragment at least 20 nt in length, for example, is intended fragments which include 20 or more contiguous bases from the nucleotide sequence of the deposited cDNA or the nucleotide sequence as shown in FIGs 1A-1B (SEQ ID NO:1).

The paragraph beginning at page 13, line 9:

By a portion of a polynucleotide of "at least 20 nt in length," for example, is intended 20 or more contiguous nucleotides from the nucleotide sequence of the reference polynucleotide (e.g., the deposited cDNA or the nucleotide sequence as shown in FIGs 1A-1B (SEQ ID NO:1)). Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3′ terminal poly(A) tract of the HOIPS I cDNA shown in FIGs 1A-1B (SEQ ID NO:1)), or to a complementary stretch of T (or U) residues, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic acid of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

The paragraph beginning at page 14, line 28:

Further embodiments of the invention include isolated nucleic acid molecules comprising a polynucleotide having a nucleotide sequence at least 95% identical, and more preferably at least 96%, 97%, 98% or 99% identical to (a) a nucleotide sequence encoding the polypeptide having the amino acid sequence in SEQ ID NO:2; (b) a nucleotide sequence encoding the polypeptide having the amino acid sequence in SEQ ID NO: 2, but lacking the N-terminal methionine; (c) a nucleotide sequence encoding the polypeptide having the amino acid sequence at positions from about 1 to about 142 in FIGs 1A-1B (SEQ ID NO:2); (d) a nucleotide sequence encoding the polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97825; (e) a nucleotide sequence encoding the mature HOIPS I polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97825; or (f) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), or (e).

The paragraph beginning at page 20, line 13:

The invention further provides an isolated HOIPS I polypeptide having the amino acid sequence encoded by the deposited cDNA, or the amino acid sequence in FIGs 1A-1B (SEQ ID NO:2), or a peptide or polypeptide comprising a portion of the above polypeptides.

The paragraph beginning at page 37, line 23:

The 5′ oligonucleotide primer has the sequence:

5′ GACTCCATGGGCGGCGGTGGGAAAGCCTG 3′ (SEQ ID NO:4) containing the underlined NcoI restriction site, which encodes 20 nucleotides of the HOIPS I protein coding sequence in FIGs 1A-1B (SEQ ID NO:1) beginning immediately after the signal peptide.

The paragraph beginning at page 37, line 28:

The 3′ primer has the sequence:

5′ GACTAGATCTGGAGCACATGATAGTAGCAT 3′ (SEQ ID NO:5) containing the underlined BglII restriction site followed by 20 nucleotides complementary to the last 20 nucleotides of the HOIPS I protein coding sequence in FIGs 1A-1B.

The paragraph beginning at page 40, line 9:

The cDNA sequence encoding the full length HOIPS I protein in the deposited clone, including the AUG initiation codon and the naturally associated leader sequence shown in FIGs 1A-1B (SEQ ID NO:2), is amplified using PCR oligonucleotide primers corresponding to the 5' and 3'

sequences of the gene. The 5' primer has the sequence 5' GAC TGGATCCGCC ATC ATG AAG GGT TTC ACA GCC AC 3' (SEQ ID NO:6) containing the underlined BamHI restriction enzyme site, an efficient signal for initiation of translation in eukaryotic cells, as described by Kozak, M., *J. Mol. Biol. 196*:947-950 (1987), followed by 20 bases of the sequence of the complete HOIPS I protein shown in FIGs 1A-1B, beginning with the AUG initiation codon. The 3' primer has the sequence 5' GACTGGTACCAG- CAGCTGCACTCTTTGGG 3' (SEQ ID NO: 7) containing the underlined, Asp718 restriction site followed by 19 nucleotides complementary to the 3' noncoding sequence in FIGs 1A-1B.

The paragraph beginning at page 47, line 13:

The DNA sequence encoding the complete HOIPS I protein including its leader sequence is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. The 5' primer has the sequence 5' GACTGGATCCGCCATCATGAAGGGTTTCACAGCCAC 3' (SEQ ID NO:6) containing the underlined BamHI restriction enzyme site followed by an efficient signal for initiation of translation in eukaryotes, as described by Kozak, M., *J. Mol. Biol. 196*:947-950 (1987), and 20 bases of the coding sequence of HOIPS I shown in FIGs 1A-1B (SEQ ID NO:1). The 3' primer has the sequence 5' GACTGGTACCAGCAGCTGCACTCTTTGGG 3' (SEQ ID NO:10) containing the underlined Asp718 restriction site followed by 19 nucleotides complementary to the non-translated region of the HOIPS I gene shown in FIGs 1A-1B (SEQ ID NO:1).

Remarks

None of the amendments adds new matter.

The amendments correct formal matters. Specifically, the amendments bring the specification into conformity with the formal drawings submitted herewith and correct the address of the ATCC.

Accordingly, Applicants respectfully request that this Amendment be entered.

Respectfully submitted,

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Version with markings to show changes made

The paragraph beginning at page 5, line 2:

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding the HOIPS I polypeptide having the amino acid sequence shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:2) or the amino acid sequence encoded by the cDNA clone deposited in a bacterial host with the American Type Culture Collection ("ATCC"), [12301 Park Lawn Drive, Rockville, Maryland 20852]Patent Depository, 10801 University Boulevard, Manassas, VA, 20110-2209, on December 16, 1996. (ATCC Deposit Number 97825).

The paragraph beginning at page 6, line 8:

[Figure 1 shows]FIGs 1A-1B show the nucleotide (SEQ ID NO:1) and deduced amino acid (SEQ ID NO:2) sequences of HOIPS I. The protein has a leader sequence of about 20 amino acid residues and a deduced molecular weight of about 17.8 kDa. The predicted amino acid sequence of the mature HOIPS I protein is also shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:2).

The paragraph beginning at page 6, line 13:

[Figure 2]FIG. 2 shows the regions of similarity between the amino acid sequences of the HOIPS I protein and chicken MD-1 (SEQ ID NO:3). The consensus sequence is shown (SEQ ID NO:17).

The paragraph beginning at page 6, line 16:

[Figure 3]FIG. 3 shows an analysis of the HOIPS I amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown. In the "Antigenic Index - Jameson-Wolf" graph, amino acid residues about 17 to about 29, about 33 to about 39, about 43 to about 52, about 56 to about 67, about 74 to about 83, about 90 to about 94, about 110 to about 120, about 125 to about 139, and about 145 to about 152 in [Figure 1]FIGs 1A-1B correspond to the shown highly antigenic regions of the HOIPS I protein. These highly antigenic fragments in [Figure 1]FIGs 1A-1B correspond to the following fragments, respectively in SEQ ID NO:2: amino acid residues about -4 to about 9, about 13 to about 19, about 23 to about 32, about 36 to about 47, about 54 to about 63, about 70 to about 74, about 90 to about 100, about 105 to about 119, and about 125 to about 132.

The paragraph beginning at page 7, line 2:

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding a HOIPS I polypeptide having the amino acid sequence shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:2), which was determined by sequencing a cloned cDNA. The HOIPS I protein of the present invention shares sequence homology with the chicken MD-1 protein. [(Figure 2)](FIG. 2) (SEQ ID NO:3). The nucleotide sequence shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:1) was obtained by sequencing the HTOCD71X clone, which was deposited on December 16, 1996 at the American Type Culture Collection, [12301 Park Lawn Drive, Rockville, Maryland 20852]Patent Depository, 10801 University Boulevard, Manassas, VA, 20110-2209. (ATCC accession number 97825) The deposited clone is contained in the pBluescript SK(-) plasmid (Stratagene, LaJolla, CA).

The paragraph beginning at page 8, line 3:

Using the information provided herein, such as the nucleotide sequence in [Figure 1]FIGs 1A-1B, a nucleic acid molecule of the present invention encoding a HOIPS I polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material. Illustrative of the invention, the nucleic acid molecule described in [Figure 1]FIGs 1A-1B (SEQ ID NO:1) was discovered in a cDNA library derived from human tonsils tissue. The gene was also identified in cDNA libraries from the following tissues: bone marrow, dendritic cells, fetal and adult brain macrophages, B cells, and lymph nodes. The determined nucleotide sequence of the HOIPS I cDNA of [Figure 1]FIGs 1A-1B (SEQ ID NO:1) contains an open reading frame encoding a protein of 162 amino acid residues and a deduced molecular weight of about 17.8 kDa. The HOIPS I protein shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:2) is about 45% identical to, and about 64% similar to, the chicken MD-1 protein [(Figure 2)](FIG. 2) in a 132 amino acid residue overlap.

The paragraph beginning at page 8, line 17:

The present invention also provides the mature form(s) of the HOIPS I protein of the present invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species on the protein. Further, it has long been known that the cleavage specificity of a secreted protein is

ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide. Therefore, the present invention provides a nucleotide sequence encoding the mature HOIPS I polypeptides having the amino acid sequence encoded by the cDNA clone contained in the host deposited with the ATCC on December 16, 1996, (ATCC Deposit No. 97825) and as shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:2). By the mature HOIPS I protein having the amino acid sequence encoded by the cDNA clone contained in the host deposited with the ATCC on December 16, 1996, (ATCC Deposit No. 97825) is meant the mature form(s) of the HOIPS I protein produced by expression in a mammalian cell (e.g., COS cells, as described below) of the complete open reading frame encoded by the human DNA sequence of the clone contained in the vector in the deposited host. As indicated below, the mature HOIPS I having the amino acid sequence encoded by the cDNA clone contained in the host deposited with the ATCC on December 16, 1996, (ATCC Deposit No. 97825) may or may not differ from the predicted "mature" HOIPS I protein shown in [Figure 1]FIGs 1A-1B (amino acids from about 1 to about 142 in SEQ ID NO:2) depending on the accuracy of the predicted cleavage site based on computer analysis.

The paragraph beginning at page 9, line 19:

In the present case, the predicted amino acid sequence of the complete HOIPS I polypeptides of the present invention were analyzed by a computer program ("PSORT") (K. Nakai and M. Kanehisa, *Genomics 14*:897-911 (1992)), which is an expert system for predicting the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated. The analysis by the PSORT program predicted the cleavage sites between amino acids 20 and 21 in [Figure 1]FIGs 1A-1B (SEQ

ID NO:2). Thereafter, the complete amino acid sequences were further analyzed by visual inspection, applying a simple form of the (-1,-3) rule of von Heinje. von Heinje, *supra*. Thus, the leader sequence for the HOIPS I protein is predicted to consist of amino acid residues -20 to -1 in SEQ ID NO:2. However, while the predicted mature HOIPS I protein consists of residues 1-142, the present inventors have identified other possible cleavage sites resulting in mature proteins having the following amino acid residues shown in SEQ ID NO:2: -7-142, -6-142, -5-142, -4-142, -3-142, -2-142, -1-142, 2-142, 3-142, 4-142, 5-142, 6-142, 7-142, 8-142, 9-142, 10-142, 11-142, 12-142, 13-142, 14-142.

The paragraph beginning at page 10, line 26:

Isolated nucleic acid molecules of the present invention include DNA molecules comprising an open reading frame (ORF) shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:1); DNA molecules comprising the coding sequence for the mature HOIPS I protein shown in [Figure 1]FIGs 1A-1B (last 142 amino acids) (SEQ ID NO:2); and DNA molecules which comprise a sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode the HOIPS I protein. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate variants.

The paragraph beginning at page 11, line 3:

In another aspect, the invention provides isolated nucleic acid molecules encoding the HOIPS I polypeptide having an amino acid sequence as encoded by the cDNA clone contained in the plasmid deposited with the ATCC on December 16, 1996 (ATCC Deposit No. 97825). In a further

embodiment, nucleic acid molecules are provided encoding the mature HOIPS I polypeptide or the full-length polypeptide lacking the N-terminal methionine. The invention also provides an isolated nucleic acid molecule having the nucleotide sequence shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:1) or the nucleotide sequence of the HOIPS I cDNA contained in the above-described deposited clone, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated molecules, particularly DNA molecules, are useful as probes for gene mapping, by *in situ* hybridization with chromosomes, and for detecting expression of the HOIPS I gene in human tissue, for instance, by Northern blot analysis.

The paragraph beginning at page 11, line 16:

The present invention is further directed to fragments of the isolated nucleic acid molecules described herein. By a fragment of an isolated nucleic acid molecule having the nucleotide sequence of the deposited cDNA or the nucleotide sequence shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:1) is intended fragments at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt in length which are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments 50, 100, 150, 200, 250, 300, 350, 400, 450, or 500 nt in length are also useful according to the present invention as are fragments corresponding to most, if not all, of the nucleotide sequence of the deposited cDNA or as shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:1). By a fragment at least 20 nt in length, for example, is intended fragments which include 20 or more contiguous bases from the nucleotide sequence of the deposited cDNA or the nucleotide sequence as shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:1).

The paragraph beginning at page 13, line 9:

By a portion of a polynucleotide of "at least 20 nt in length," for example, is intended 20 or more contiguous nucleotides from the nucleotide sequence of the reference polynucleotide (e.g., the deposited cDNA or the nucleotide sequence as shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:1)). Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3′ terminal poly(A) tract of the HOIPS I cDNA shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:1)), or to a complementary stretch of T (or U) residues, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic acid of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

The paragraph beginning at page 14, line 28:

Further embodiments of the invention include isolated nucleic acid molecules comprising a polynucleotide having a nucleotide sequence at least 95% identical, and more preferably at least 96%, 97%, 98% or 99% identical to (a) a nucleotide sequence encoding the polypeptide having the amino acid sequence in SEQ ID NO:2; (b) a nucleotide sequence encoding the polypeptide having the amino acid sequence in SEQ ID NO: 2, but lacking the N-terminal methionine; (c) a nucleotide sequence encoding the polypeptide having the amino acid sequence at positions from about 1 to about 142 in [Figure 1]FIGs 1A-1B (SEQ ID NO:2); (d) a nucleotide sequence encoding the polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97825; (e) a nucleotide sequence encoding the mature HOIPS I polypeptide having the

amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97825; or (f) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), or (e).

The paragraph beginning at page 20, line 13:

The invention further provides an isolated HOIPS I polypeptide having the amino acid sequence encoded by the deposited cDNA, or the amino acid sequence in [Figure 1]FIGs 1A-1B (SEQ ID NO:2), or a peptide or polypeptide comprising a portion of the above polypeptides.

The paragraph beginning at page 37, line 23:

The 5′ oligonucleotide primer has the sequence:

5′ GACTCCATGGGCGGCGGTGGGAAAGCCTG 3′ (SEQ ID NO:4) containing the underlined NcoI restriction site, which encodes 20 nucleotides of the HOIPS I protein coding sequence in [Figure 1]FIGs 1A-1B (SEQ ID NO:1) beginning immediately after the signal peptide.

The paragraph beginning at page 37, line 28:

The 3′ primer has the sequence:

5′ GACTAGATCTGGAGCACATGATAGTAGCAT 3′ (SEQ ID NO:5) containing the underlined BglII restriction site followed by 20 nucleotides complementary to the last 20 nucleotides of the HOIPS I protein coding sequence in [Figure 1]FIGs 1A-1B.

The paragraph beginning at page 40, line 9:

The cDNA sequence encoding the full length HOIPS I protein in the deposited clone, including the AUG initiation codon and the naturally associated leader sequence shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:2), is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. The 5' primer has the sequence 5' GAC TGGATCCGCC ATC ATG AAG GGT TTC ACA GCC AC 3' (SEQ ID NO:6) containing the underlined BamHI restriction enzyme site, an efficient signal for initiation of translation in eukaryotic cells, as described by Kozak, M., *J. Mol. Biol. 196*:947-950 (1987), followed by 20 bases of the sequence of the complete HOIPS I protein shown in [Figure 1]FIGs 1A-1B, beginning with the AUG initiation codon. The 3' primer has the sequence 5' GACTGGTACCAG- CAGCTGCACTCTTTGGG 3' (SEQ ID NO: 7) containing the underlined, Asp718 restriction site followed by 19 nucleotides complementary to the 3' noncoding sequence in [Figure 1]FIGs 1A-1B.

The paragraph beginning at page 47, line 13:

The DNA sequence encoding the complete HOIPS I protein including its leader sequence is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. The 5' primer has the sequence 5' GACTGGATCCGCCATCATGAAGGGTTTCACAGCCAC 3' (SEQ ID NO:6) containing the underlined BamHI restriction enzyme site followed by an efficient signal for initiation of translation in eukaryotes, as described by Kozak, M., *J. Mol. Biol. 196*:947-950 (1987), and 20 bases of the coding sequence of HOIPS I shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:1). The 3' primer has the sequence 5' GACTGGTACCAGCAGCTGCACTCTTTGGG 3' (SEQ ID NO:10) containing the underlined Asp718 restriction site followed by 19 nucleotides

complementary to the non-translated region of the HOIPS I gene shown in [Figure 1]FIGs 1A-1B

(SEQ ID NO:1).